Searching for the Achilles' heel of melanoma cells: new treatment modalities

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Metastatic melanoma is almost always fatal and represents a great challenge for cancer therapies because of its notorious resistance to chemotherapy. Both, standard chemotherapeutic agent DTIC, as well as adjuvant immunotherapies with interferon-α or interleukin-2 produce limited response rate of 10–20% and only modestly improve the outcome in patients with metastatic melanoma. Considerable efforts have been made over the last 2 decades in pursuit of new treatment modalities and identification of molecular events associated with melanoma development and progression. After the initial publication in 2002 by Davies et al., who reported a high rate of occurrence of somatic mutations in BRAF serine/threonine kinase gene in human melanoma, numerous in vitro and animal studies confirmed the therapeutic value of inhibiting BRAF-MEK1/2-MAPK pathway. However, a single-agent therapy using clinically approved BRAF/VEGFR-2 inhibitor, Sorafenib (BAY43-9006; Nexavar, Bayer Pharmaceuticals Corp., West Haven, CT, USA) demonstrated clinical ineffectiveness (Eisen et al., 2006). Encouragingly, analyses of Phase II clinical studies continue to demonstrate an increase in progression free-survival in the Sorafenib plus chemotherapy combination groups as compared to placebo plus chemotherapy (McDermott et al., 2008). However, in 2006, Bayer (the maker of Sorafenib) announced the results of a phase III clinical trial in patients with advanced melanoma, in which the combination of Sorafenib with carboplatin plus paclitaxel has failed to show superiority in progression-free survival (Lejeune et al., 2007).

Sorafenib, however, is a cRAF and VEGFR-2 inhibitor and is not specific to the BRAF (V600E) mutation found in a large proportion of melanomas. This led Plexxikon, Inc. to develop a new selective inhibitors of BRAF (V600E), PLX4720 and PLX-4032. The preclinical studies with these compounds showed inhibition of tumor growth and metastasis of only melanoma cells harboring the V600E mutation but not cells with wild-type BRAF. Clinical trials with these compounds are now underway with much anticipation by the melanoma clinical community. One lesson learned from the Sorafenib trials is that using RAF/MEK inhibitors as single agents or in combination with chemotherapy may not be sufficient or effective in inhibiting melanoma growth and metastasis. Therefore, the notion that targeting more than one pathway controlling melanoma growth is now very well accepted. For example, in addition to BRAF–MAPK pathway, PI3K-Akt-mTOR pathway is frequently upregulated in melanoma through genetic alteration and serves as a possible early marker of melanoma progression. It has been proposed that simultaneous inactivation of both kinase pathways must be achieved in order to produce significant clinical effect in melanoma (Lejeune et al., 2007). Indeed, combination of rapamycin (mTOR inhibitor) and Sorafenib or anti-VEGFR-2 antibody bevacizumab produced synergistic inhibition of melanoma cell proliferation (Molhoek et al., 2005, Molhoek et al., 2008). In their latest study, Molhoek et al. (2008) demonstrated that bevacizumab alone induced inhibition of proliferation of VEGFR-2 positive but not VEGFR-2 negative melanoma cells, without inducing cell apoptosis. However, apoptosis was ensued when bevacizumab was combined with rapamycin. This study demonstrates an existence of an autocrine growth loop in VEGFR-2 positive melanoma and a potential for nonangiogenic mechanism of action of bevacizumab.

Constitutive activation of Akt protein kinase has been linked to activation of NFκB signaling pathway – a hallmark of tumor chemoresistance. Recently, Schön et al. (2008) reported on the development of a novel NFkB inhibitor KINK-1 (kinase inhibitor of NF-κB-1) that belongs to a new class of small-molecule compounds, which specifically inhibits IKKβ (a subunit of IκB kinase complex). KINK-1 effectively reduced constitutive and TNFα-induced activity of NFkB and downregulated the expression of downstream target genes in 14 melanoma cell lines. Although KINK-1 alone produced very low antiproliferative and proapoptotic effect, it nevertheless inhibited cell proliferation and induced apoptosis in melanoma cells when combined with suboptimal doses of cytostatic doxorubicin, camptothecin and tamoxifen (Schön et al., 2008). Likewise, the apoptotic response was

Coverage on: Molhoek et al. (2008). Human melanoma cytolysis by combined inhibition of mammalian target of rapamycin and vascular endothelial growth factor/vascular endothelial growth factor receptor-2. *Cancer Res.* 68: 4392–4397.

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Abbas et al. (2007). Preclinical studies of celastrol and acetyl isogambogic acid in melanoma. *Clin. Cancer Res.*13: 6769–6778.

Madhunapantula et al. (2008). PBISe, a novel selenium-containing drug for the treatment of malignant melanoma. *Mol. Cancer Ther.* 7: 1297–1308.

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Lovat et al. (2008). Increasing melanoma cell death using inhibitors of protein disulfide isomerases to abrogate survival responses to endoplasmic reticulum stress. *Cancer Res.* 68: 5363–5369.

Giammarioli et al. (2008). Pyrimethamine induces apoptosis of melanoma cells via a caspase and cathepsin double-edged mechanism. *Cancer Res.* 68: 5291–5300.

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increased when the natural death receptor ligand TRAIL or the CD95-activating CH-11 mAb was combined with KINK-1. Finally, in a B16F10 model of mouse melanoma pulmonary metastasis, mice treated with combination of doxorubicin and KINK-1 exhibited markedly fewer metastasis and significantly less tumor cell proliferation in situ when compared to either treatments alone. This study offers proof of the concept for utilizing IKKβ inhibitors in melanoma. Elucidating a complete spectrum of KINK-1 action on the molecular pathways defining cellular resistance may offer additional approaches that will help to augment therapeutic efficacy of this novel drug. By being specific inhibitor of NFκB, KINK-1 may have advantage over the non-specific NFκB inhibitor of proteasome bortezomib (velcade). However, it is still unclear whether the usage of IKKβ inhibitors will not interfere with other physiological pathways in normal cells.

Endogenous nitric oxide (NO) production and the expression of the inducible nitric oxide synthase (iNOS) have been implicated in the chemoresistance of melanoma (Ekmekcioglu et al., 2000). The development of the new compound, PBISe, that targets simultaneously the iNOS and the Akt has been reported by the group of Robertson (Madhunapantula et al., 2008). PBISe was synthesized as a selenium-containing isosteric analogue of an iNOS inhibitor PBIT, and demonstrated to have >10-fold higher efficacy than PBIT in killing cultured melanoma cells. PBISe caused decrease in melanoma cell proliferation, and increase in apoptosis. Inhibition of cellular proliferation mediated by PBISe was associated with a G2-M phase cell cycle block and linked to excessively high MAPK activity causing decreased cyclin D1 and increased p21 as well as p27 levels. This causal involvement of MAPK in inhibition of cell proliferation remains, however, to be confirmed experimentally. Meanwhile, the pro-apoptotic effect of PBISe was linked to inhibition of Akt3 signaling, elevating cleaved caspase-3 and PARP levels. Finally, when tested on 6-day-old 1205 and UACC903 melanoma xenografts, PBISe, but not PBIT, showed significant inhibition of tumor growth by 30–50% with negligible associated systemic toxicity (Madhunapantula et al., 2008). Collectively, these results suggest that PBISe is a potent chemotherapeutic agent with novel properties enabling the targeting of iNOS, Akt3, and MAPK signaling, thereby promoting melanoma cell apoptosis and inhibition of proliferation.

The group of Ronai has identified two new small molecule compounds that mimic the toxic effect of activating transcription factor 2 (ATF2)-targeting peptide on melanoma cells (Abbas et al., 2007). From their stepwise chemical library screen, celastrol (CSL) and acetyl isogambogic acid (AIGA) have emerged as the two compounds capable of inducing apoptosis in mouse and human melanoma cells, inhibition of ATF2-driven transcription, and induction of JNK kinase and AP1/c-Jun transcriptional activity. This group also generated two ester derivatives of CSL with higher potency and demonstrated that all three compounds significantly attenuated the growth of both mouse and human melanoma cells *in vivo* using immunocompetent and immunodeficient mouse models. Furthermore, CSL and CA19 were efficient in inhibiting pulmonary human melanoma metastasis in a nude mouse model, suggesting that these compounds should be considered as a possible modality for melanoma cancer therapy (Abbas et al., 2007).

Several new studies have emerged recently aiming at exploiting apoptotic pathways as yet another modality for melanoma treatment. Targeting the expression of ER stress chaperons such as those with protein disulfide isomerase (PDI) activity can attenuate chemotherapy-induced ER stress responses and tip the balance towards apoptosis in stressed tumor cells. Exploiting this notion, Lovat et al. (2008) demonstrated that PDI inhibitor bacitracin potentiated stress response and apoptosis in cultured melanoma cells treated with the novel chemotherapeutic drugs fenretinide and velcade. Overexpression of the main cellular PDI, P4HB in its wild-type but not mutant form, abrogated the apoptosis-enhancing effect of bacitracin. These results suggested that the small-molecule PDI inhibitors have significant potential as a powerful tool for enhancing the efficacy of chemotherapy in melanoma.

Last but not least, the group of Pierdominici published results of a preclinical evaluation of pirymethamine, an antifolate drug typically used in the treatment of infections caused by protozoan parasites (Giammarioli et al., 2008). The authors demonstrated that pirymethamine induced apoptosis in metastatic melanoma cells via the activation of the cathepsin B and the caspase cascade (i.e. caspase-8 and caspase-9), subsequent mitochondrial depolarization, as well as inhibition of Bcl-2. Pyrimethamine also induced a marked inhibition of cell growth and an S-phase cell cycle arrest. Such multifaceted mechanism of action, in which different inducers or regulators of apoptosis are simultaneously implicated, may indeed allow for the death defects of melanoma cells to be bypassed. Consequently, the growth of subcutaneously injected into SCID mice metastatic melanoma cells was inhibited by pyrimethamine (Giammarioli et al., 2008).

Several attempts have been made in the last year to develop antibodies or small molecules targeting several pathways that control melanoma growth and metastasis. It is now becoming clear that targeting one pathway is insufficient. Finding the right combination remains yet the real challenge for both basic scientists and clinicians.

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